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Novel RNAi Therapy – Intron-Derived MicroRNA Drugs

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Abstract: MicroRNAs (miRNAs) are phylogenetically widespread small RNAs in animals and plants; the origin and function of these molecules in drug design is unknown. Our recent findings of small regulatory RNAs in non-coding regions of the human genome indicate a new strategy for modern drug designs. A novel gene modulation system was found within mammalian introns, regulating intracellular gene transcripts homologous to certain 5'-proximal regions of a native intron. These regions are normally located between the 5'-splice site and the next branch-point domain. Using Pol-II-directed expression and splicing excision of an artificial intron with various 5'-proximal synthetic inserts, we have successfully identified the generation of intron-derived microRNA-like molecules (Id-miRNA) from these regions as a tool for analysis of gene function and development of gene-specific therapeutics. The Id-miRNAs in hairpin conformations deliver maximal RNAi-related gene silencing effects on target genes in several mammalian cells. Based on the diversity of known miRNA structures and the complexity of genomic non-coding regions, the Id-miRNA generation mechanism may lead to one of the major gene modulation systems for developmental regulation, intracellular immunity, heterochromatin inactivation and genomic evolution in eukaryotes.

Keywords: Pol-II-directed RNA interference (RNAi), microRNAs (miRNA), splicing-competent intron (SpRNAi), intron-derived miRNA (Id-miRNA).

MICRORNA (MIRNA)

The ribonucleic acid (RNA), an intermediate between genome and protein, has been thought to be a biological connection between a genetic code and its cellular function. The gene is primarily transcribed as the pre-mRNA, which is a long, single-stranded RNA molecule containing both protein-coding exons and non-coding introns. The introns must be removed by splicing processes and the remaining exons are ligated together to form a mature mRNA for protein synthesis. This reduction in length of the pre-mRNA by RNA splicing involves spliceosome complexes of five small nuclear ribonucleoprotein particles (U1, U2, U4-U6 snRNPs) and a large number of non-snRNP splicing factors. During splicing, the snRNPs interact with several consensus domains of the intron, including a 5'-splice site, an interior branch-point domain, a poly-pyrimidine tract and a 3'-splice site. However, recent studies demonstrated that numerous small RNAs derived from non-coding regions of the genome are untranslated and play important regulatory roles in numerous biological processes, including developmental regulation [1, 2], gene modulation [3-7], intracellular defense [8, 9], heterochromatin imprinting [10, 11, 12] and genomic evolution [13].

The small non-coding RNAs consist of two structurally distinctive categories: double-stranded short interfering RNAs (siRNA) and single-stranded microRNA (miRNA)-like molecules. The siRNA functions in gene silencing, interfering with intracellular expression of genes with almost complete complementarity [14, 15], whereas miRNA-like molecules trigger either posttranscriptional or transcriptional regulation depending on the degree of complementarity with their target genes [10, 16]. Most of miRNA-like molecules were native transcripts in various eukaryotes, ranging from yeast (*Schizosaccharomyces spp.*) [17], plant (*Arabidopsis spp.*) [18-20], nematode (*Caenorhabditis elegans*) [2-6], fly (*Drosophila melanogaster*) [21, 22], mouse [13, 23] to human [7, 13, 24-26]. To this day, the miRNA-like molecules were given different names, including small temporal RNAs (stRNA) [3-5], small hairpin RNAs (shRNA) [27], tiny noncoding RNA (tncRNA) [28, 29] and spliced intron fragments (spRNA) [13]. These molecules share several structural and mechanical similarities although there are some species-specific variations in their secondary conformations and biological functions. First, they are single-stranded small RNAs derived from non-coding RNA transcripts with high secondary structures such as hairpins and stem-loops. Second, the active forms of these molecules are produced by Dicer-like RNase processing. Third, these molecules provide either transcriptional or posttranscriptional functions, depending on their complementarity to a gene target. Last, the functions of these molecules rely on accessibility of the relative RNAi effector complexes, *e.g.* RNA-induced silencing complex (RISC) around the molecules. Although siRNAs are derived from a large double-stranded RNA (dsRNA) template, the resulting antisense strand RNA fragments in the RISC can be considered as an active form of miRNAs.

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ROLE OF MIRNA IN GENE REPRESSION

The miRNA was originally defined as a class of small non-coding regulatory RNAs, sized about 20 base nucleotides, that impair translation by imperfect base-pairing to target mRNAs. Novel gene modulation technology was recently developed involving miRNA generation, which is associated with an intracellular defense system, to eliminate undesired transgenes and foreign RNAs, including viral infections and retrotransposon activities [8, 30]. Most recently, a novel gene modulation system was found within mammalian introns, regulating intracellular gene transcripts homologous to certain 5'-proximal regions of a native intron. These regions have been localized between the 5'-splice site and the next branch-point domain, which are not the binding sites of snRNPs during RNA splicing and processing [13]. Based on this information, novel therapeutics directly against cancers and viral infections were designed and tested [7, 8]. However, their transcriptional products remain to be elucidated.

This novel application of miRNAs in drug design is consistent with recent research in the genomes of *C. elegans* and human that discovered a new kind of miRNA-like hairpin RNAs located within the non-protein-coding regions (*i.e.* introns) of certain genes [12, 24, 28]. Using artificial introns with various 5'-proximal hairpin inserts, we have successfully demonstrated the involvement of RNA polymerase type-II (Pol-II) and RNA splicing machineries in this intron-derived miRNA generation mechanism in rat and human cells [7, 13]. It has been reported that a coupled interaction of nascent Pol-II pre-mRNA transcription and intron excision within a certain region proximal to genomic DNA (*i.e.* perichromatin fibrils) resulted in potential intracellular location for generating miRNA [31-34]. After the processing of the pre-mRNA, the mature mRNA and numerous spliced introns are produced. Some of the spliced introns become aberrant RNAs, which are further cleaved by Dicer-like RNase or complementing-repair complex to form miRNAs (Fig. **1**). After splicing, about 10~30% of the introns were found in the cytoplasm with a moderate half-life that is long enough to execute additional functions [35]. Indeed, the intron-derived miRNAs (Id-miRNA) were capable of triggering either translation repression or mRNA degradation depending on the degree of complementarity and homology with their target genes.

INTRON-DERIVED MIRNAS (ID-MIRNA)

The artificial introns we constructed for Id-miRNA generation mimic the native structure of a pre-mRNA intron [13]. As shown in (Fig. **2**), this splicing-competent artificial intron, SpRNAi, is flanked with a 5'-splice site (5'SS) and a 3'-splice site (3'SS) and contains a branch-point domain (BrP), a poly-pyrimidine tract (PPT) and at least one intronic insert. The inserted oligonucleotide sequence located between

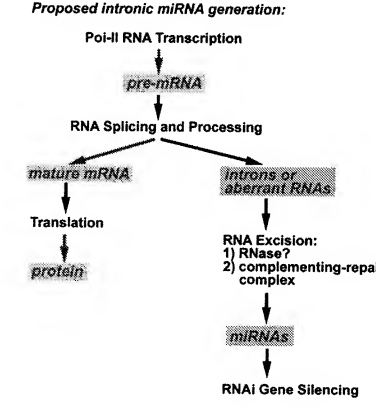

Fig. (1). Proposed model for intron-derived miRNA (Id-miRNA) generation from pre-mRNA transcription and splicing processes.

5'SS and BrP is designed to be either homologous or complementary, or both, to a targeted gene exon. This portion of the intron would normally represent a 5'-proximal region unrecognized by snRNPs during RNA splicing and processing. To prevent inaccuracy or failure of RNA splicing, the SpRNAi contains 3'-transcription and translation stop codons (Ts), which direct splicing-defective pre-mRNAs to be degraded by the non-sense mediated decay (NMD) pathway. For intracellular expression, the SpRNAi must be co-expressed with a gene driven by Pol-II transcription from an RNA promoter (P). We thus incorporated the SpRNAi construct into an intronless *HcRed1* red fluorescent protein (*rGFP*) gene vector to form a recombined SpRNAi-*rGFP* gene, of which the original *rGFP* fluorescent structure was disrupted by the intron insertion. After the mRNA maturation processes of Pol-II transcription and then intron splicing, the reappearance of red fluorescent emission in the cells transfected by the SpRNAi-*rGFP* gene was observed.

We have tested various SpRNAi-*rGFP* genes in several mammalian cells, and observed that the maturation of such intron-inserted *rGFP* gene transcripts triggers strong suppression of genes homologous to 5'-proximity of the intron sequence. The splicing and processing of the introns containing inserts in either sense or antisense conformation produced equivalent gene silencing effects, while that of a palindromic hairpin insert containing both sense and antisense strands resulted in synergistic maximal effects. We further detected that the sizes of these splicing-processed intron fragments are ranged about 15–45 base nucleotides (nt) approximate to the newly identified intronic miRNAs in *C. elegans.* According to the variety and complexity of native miRNA structures, this is the first evidence that miRNAs are able to be produced intracellularly through the intronic splicing and processing mechanisms.

POTENTIAL MECHANISMS OF ID-MIRNA

The mechanism(s) by which intronic miRNA represses gene expression is unclear. We postulated that homologous exchange between intronic RNAs and genomic DNAs accounted for its specific gene silencing effects [8, 13]. The [P^{32}]-labeled DNA component of an mRNA-DNA duplex construct in cell nuclear lysates was intact during the effective period of miRNA-induced RNA interference (RNAi) phenomena, while the labeled RNA part was replaced by cold homologues and degraded into small RNAs within a 3-day incubation period (Fig. **3A**). These observations indicated the possibility that degraded RNA fragments facilitate the degradation of homologous parts of the target mRNAs (Fig. **3B**). Alternatively, the newly recombined mRNA component of the mRNA-DNA construct is further processed by Pol-II and an unknown RNA excision machinery is involved to generate more miRNAs for long-term gene silencing. Mammalian Pol-II has been reported to

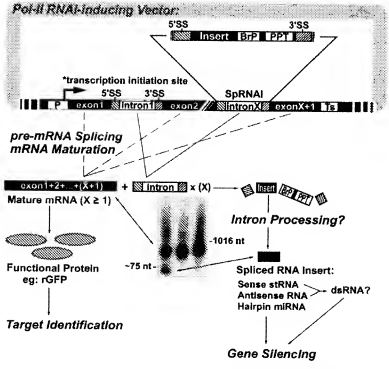

Fig. (2). Experimental evidences for mammalian Id-miRNA generation. Artificial introns, SpRNAi, were excised out of a vector-based co-expressed gene, *rGFP,* and processed into miRNA-like small RNAs for silencing the genes either homologous or complementary to the small RNAs. The low-stringent northern blot analysis (middle bottom) showed the generation of mature *rGFP* mRNA (~900 nt) and the miRNA-like molecules (15–45 nt). The release of small RNA fragments was observed only from the spliced gene transcripts (left), but not from an intronless *rGFP* mRNA (middle) or a splicing-defective SpRNAi-*rGFP* pre-mRNA (right), indicating the requirement of RNA splicing/processing machineries for intronic miRNA generation. There is an unknown mechanism for processing a minimal 90–nt spliced intron to small Id-miRNAs.

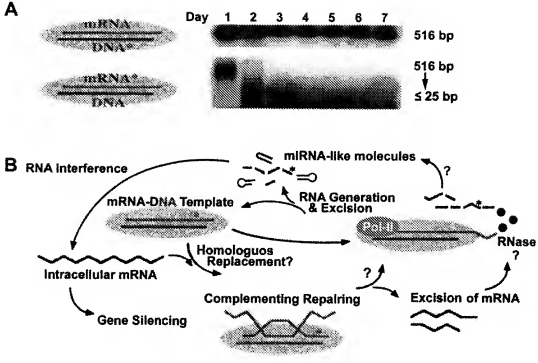

Fig. (3). Involvement of Pol-II and RNA excision in intron-mediated RNAi gene silencing. The Id-miRNA generation is tested using the long template of mRNA-DNA hybrid constructs, mimicking the interaction between nascent mRNA and genomic DNA. A, the replacement of RNA component in an mRNA-DNA hybrid was found to correlate with the generation of miRNA-like molecules, while the DNA component remains intact and attached to the RNA component. B, a proposed model of the miRNA-induced RNAi mechanism contains a systemic interaction among Pol-II transcription, RNA excision and probably complementing-repairing machineries. The resulting miRNA-like molecules are able to silence intracellular RNA homologues and thus deliver the RNAi effects.

possess RNA-dependent RNA polymerase (RdRp) activities [36, 37]. Further, *p58/HHR23*, a transcriptional protein that codes for xeroderma pigmentosum group C (XPC)-associated excision repair of aberrant nucleotides, was identified as a candidate helicase for the RNA excision machinery [13]. Therefore, the function of *p58/HHR23* may be similar to the RNA helicase activities of *Mut6* in *Chlamydomonas* or *Mut14* in *C. elegans*, which is responsible for RNA unwinding in RNAi-related gene silencing and necessary for the splicing of pre-mRNAs [38, 39]. Thus, it is highly likely that Pol-II RNA excision directs both mRNA-DNA-derived and intron-derived small RNA generation, resulting in single-stranded small RNAs of about 20 nt comparable to the general sizes of Dicer-processed miRNA intermediates usually observed in the regulations of developmental events. Moreover, intron-derived miRNAs and Dicer-processed miRNA intermediates isolated by guanidinium-chloride ultracentrifugation elicit strong, but short-term gene silencing effects on the homologous genes in transfected cells, indicating that both types of RNAs are capable of inducing RNAi effects. Since such miRNAs can be constitutively derived from the large template of an mRNA-genomic DNA complex, the long-term effect of the Id-miRNA-induced RNAi phenomena is maintained by the accumulation of sufficient Id-miRNAs rather than due to their stability. These findings also explain the delayed initiation phenomena observed previously in intron splicing-mediated gene silencing mechanisms [7, 13].

ID-MIRNA AND RNAI

We developed a therapeutic miRNA-inducing vector based on the observation that Pol-II-directed miRNA-induced RNAi effects have improved upon several outcomes of Pol-III-based siRNA systems [8, 13]. In mammals, interferon-induced non-specific RNA degradation results in repression of the specificity of dsRNA-mediated RNAi effects when the dsRNA size is larger than 30 bp or its concentration is high [40-42]. Such a cellular response usually triggers global, non-specific mRNA degradation and may cause strong cytotoxic effects in the cells when the levels of interferon are high. Pol-III has been reported to transcribe long RNAs, hence, the Pol-III systems could generate dsRNAs longer than the expected size [43-45]. The effect of dsRNA-induced interferon has been frequently used as one of the most effective adjuvant therapies to direct cancer cell suicide. Furthermore, the Pol-III-based vector delivery of siRNA expression for gene therapy *in vivo* is not practical because the incompatibility between the Pol-III promoter and another RNA promoter in the delivery vector or from endogenous viruses [42, 46]. These safety issues will be great challenges for using multiple RNA promoter-driven expression systems, which are likely to disturb the global balance of intracellular gene regulations. Conversely, Pol-II-based miRNA-inducing vectors offer the advantages of low dosage, high potency, long-term efficacy, and lack of overt toxicity for both *in vitro* and *in vivo* applications as demonstrated previously [8, 13]. Since intron-derived miRNAs (Id-

miRNA) must be co-expressed with a gene containing the intron, such an miRNA generation process is completely dependent on the intracellular regulations of Pol-II RNA transcription and splicing of the gene. Thus, the levels of Id-miRNA-induced gene modulation in cells are well regulated by the activity of the RNA promoter of the co-expressed gene. To make sure that the vector is safe, the Id-miRNA sequence is inserted into the non-regulatory region (mostly the 5'-proximal non-snRNP-binding area) of a native gene intron for co-expression and co-regulation along with the gene of interest. The intron insertion may be performed through homologous recombination or transposon integration, but is not necessarily required to use the promoter of the viral vectors. This novel RNA interference approach may provide an alternative gene therapy in combating cancers and viral infections, and is currently applied to the studies of cancer research, developmental biology and anti-viral drug development.

To identify the intracellular location of Id-miRNA generation, a recombined SpRNAi-*rGFP* gene, schemed out in (Fig. **2**), was shown to regain its red fluorescent emission on the membranes of transfected cells after successful expression and excision of the inserted intronic miRNAs (Fig. **4A**). There is no homology or complementarity between the SpRNAi-*rGFP* gene and its expression vectors. Upon transfection of SpRNAi-*rGFP* genes containing various synthetic intronic insert sequences homologous to a target exon, the hairpin-like homologues resulted in maximal gene silencing efficacy. For example, the transfection of SpRNAi-*rGFP* genes targeting the 279-303 nt open-reading-frame region of *Aequorea victoria* green fluorescent protein (*eGFP*) was demonstrated to be highly significant in silencing eGFP protein expression. The use of *eGFP*-positive HCN-A94-2 rat neuronal stem cells offered an excellent visual aid to observe the decreased green fluorescent emission of *eGFP* in the red fluorescent *rGFP*-expressing cells [47]. Silencing of *eGFP* was detected at 42–48 hours after transfection, indicating a potential requirement for precise timing of the production of sufficient small interfering intron-inserts from the SpRNAi-*rGFP* gene. Quantitative knockdown levels of eGFP protein (27 kDa) were also shown to be significantly altered (Fig. **4B**), with reduction rates of 56 ± 6% for the transfection of inserts homologous to the sense strand of the *eGFP* target (sense-*eGFP*), 50 ± 4% for that of the antisense strand of the *eGFP* target (antisense-*eGFP*) and 81 ± 2% for that of hairpin inserts containing both strands of the eGFP target (hairpin-eGFP). No knockdown specificity to *eGFP* was detected by the transfection of intron-free *rGFP* gene (*rGFP*(–)), or SpRNAi-*rGFP* gene containing hairpin inserts homologous to either *integrin* β*1* exon 1 (hairpin-*ITGb1* mock control) or to HIV-1 *gag*-p24 gene (hairpin-HIV p24 negative control). These findings suggest that the Id-miRNA gene silencing is determined by the homology and complementarity of the intronic insert to the targeted gene transcript, regardless of the insert orientation.

In low-level eukaryotes, miRNA represses mRNA translation usually producing no significant mRNA destruction, whereas the siRNA mainly triggers mRNA degradation. Apart from the stringent complementarity of siRNA to its mRNA targets, miRNAs are originated from single-stranded long RNA and paired with target mRNAs that have partial complementarity to them such as small RNAs in hairpin constructs. Since the miRNA-induced gene silencing is most efficiently at the level of protein synthesis and the physiological response of cells is mainly dependent on protein function, the Id-miRNA-induced gene silencing at the protein level provides strong support for the functional and physiological significance of miRNA-associated RNAi effects. In addition, similar results were observed in human prostatic cancer LNCaP cells using SpRNAi inserts that target 244-265 nt region of *integrin* β*1* gene (*ITGb1*; 29 kDa) (Fig. **4C**). The expression levels of ITGb1 protein were significantly reduced by the transfection of SpRNAi-*rGFP* genes containing sense-*ITGb1* (52 ± 2% decrease), antisense-*ITGb1* (51 ± 3% decrease) and hairpin-*ITGb1* (86 ± 1% decrease)inserts ($n = 3$, $p < 0.01$). No knockdown specificity was detected by the transfection of either intronless *rGFP* gene (blank control) or SpRNAi-*rGFP* gene containing hairpin-HIV *gag*-p24 insert (negative control). These findings suggest that the Id-miRNA-mediated gene silencing mechanism is a universal cellular response to intronic sequences with homology or complementarity to existing exons in cells. Because of the diversity of miRNA structures and the complexity of genomic introns, further investigation of the mechanism by which cells process a minimal 90 nt intron sequence into small Id-miRNA molecules may shed light on not only a novel intracellular defense system but also a major gene regulation system in mammals.

ID-MIRNAS AS ANTIVIRAL AGENTS

We have demonstrated that the Id-miRNA-induced RNAi had a great potential as for the prevention and treatment of HIV-1 infection [48]. We examined the effects of silencing HIV-mediated cellular genes in HIV-infected $CD4^+$ T lymphocytes. The CD4 molecule functions in antigen-specific T-cell activation, however, after the introduction of foreign transgenes, the HIV-mediated cell responses to the activation of T-cells can not be analyzed *in vivo* due to the variable viral infectivity and infection rates in AIDS patients. We used *in vitro* and *ex vivo* systems for this series of studies. In a short-term $CD4^+$ T cell culture *ex vivo* that HIV infectivity and infection rates were normalized, differentially expressed genes between HIV^- and HIV^+ human $CD4^+$ lymphocytes in both acute (< 2 weeks) and chronic (> 2 years) infection phases were analyzed by microarray technologies [8, 48]. Three targets, *Nef*-associated *Naf1*β, *Nb2* homologous protein to *Wnt-6* (*Nb2HP*) and *Tax1* binding protein (*Tax1BP*) were identified to show high potency in inhibiting HIV-1 infectivity. Fig. **5A** shows the silencing of a single gene slightly suppresses HIV-1 infection, reducing the total viral load to a maximal 70% of that of the controls. This low rate of reduction is probably due to the partial role of each individual target gene plays in AIDS-related complications. Indeed, the silencing effects of all three genes showed a much higher additive reduction of HIV-1 infection (19% of the controls) with no overt detection of T cell death. Protein levels of the viral *gag*-p24 marker (Fig. **5B**) were similarly reduced. These findings suggest that the Id-miRNA-derived RNAi gene silencing is capable of repelling foreign transgenes through concurrent knockout of multiple gene interactions and therefore point to

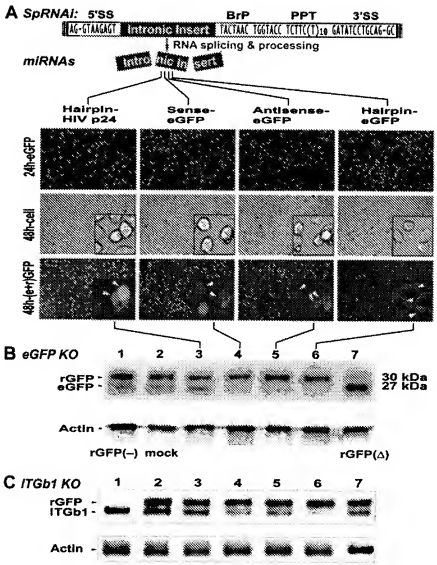

Fig. (4). Strategy for analysis of Id-miRNA-derived RNAi effects using artificial introns. A constitutive Id-miRNA expression system was generated by recombination of an artificial intron, SpRNAi into an *rGFP* gene. A, after co-expression of the red *rGFP* gene and intronic miRNAs directed against green *eGFP* expression, different gene silencing effects were detected corresponding to the miRNA conformations as shown by knockdown potency as: hairpin-insert >> sense-strand ≈ antisense-strand >> mock control. There is no visual difference at 24–h post-transfection, whereas the ratio of *eGFP* to *rGFP* fluorescent emission was significantly changed 48–h post-transfection, reflecting a lag period of initiation in the transition of *eGFP* to *rGFP*. A close-up view of *rGFP*-transfected cells (white arrows) is provided in the lower small windows. B, Western analysis of the RNAi effects on eGFP further confirmed the results of A. C, the same approach for silencing *integrin β1 (ITGb1)* in human LNCaP cancer cells was also found to be consistent with the findings of A and B, indicating that intron-mediated RNAi is most likely to be a global phenomenon for gene regulation in mammals.

a useful strategy for the development of viral miRNA vaccination and therapy.

CONCLUSIONS

The novel Pol-II-directed generation of Id-miRNA reveals a complicated intracellular network for defending cells against undesired transgenes in probably all eukaryotes. This systemic network involves interactions among pre-mRNA transcription, RNA splicing-processing, and most likely complementing-repairing machineries. Our current findings may shed light on the mechanism by which the interactive pathway takes place. First, we discovered that an mRNA-DNA hybrid template greater than 300 bp can trigger the posttranscriptional silencing of *bcl-2* oncogene expression to re-sensitize drug-resistant prostate cancer cells to the apoptotic effect of a phorbol ester drug [7]. The involvement of Pol-II was identified to play a potential role of RdRp in

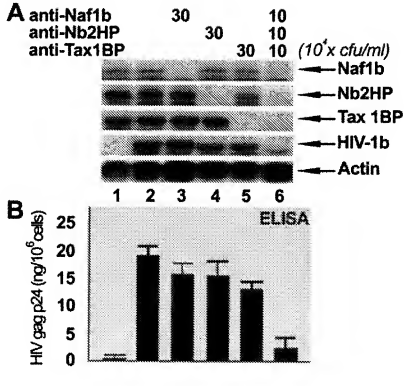

Fig. (5). *Ex vivo* trials on HIV-1 vaccination using Id-miRNA transfection directed against cellular genes. A, the Id-miRNA-induced RNAi effects were tested on three AIDS-facilitating cellular genes: *Naf1*β (AJ011896), *Nb2HP* (H12458) and *Tax1BP* (U25801). The combined effect of all three gene knockdowns was sufficient to prevent HIV-1 infection in $CD4^+$ T lymphocytes extracted from peripheral blood mononuclear cells of HAART-experienced donors (lane 6). Northern blot analyses were shown from left to right: (1) normal T cells without HIV-1 infection (blank control); (2) HIV-1-infected T cells (positive control); (3) anti-*Naf1b* SpRNAi treatment of (2); (4) anti-*Nb2HP* SpRNAi treatment of (2); (5) anti-*Tax1BP* SpRNAi treatment of (2); and (6) combinational treatment of (3), (4) and (5). B, Bar chart displays the ELISA results of HIV-1 *gag*-p24 protein expression corresponding to each treatment of A.

this gene silencing mechanism. Second, the same approach was used in embryonic chickens to successfully knock down one of critical organ morphogens, β-*catenin*, and thus create a localized transgenic silencing effect *in vivo* [8]. Then, the treatment of anti-HIV Id-miRNAs was proven to be effective and safe in T cells *ex vivo*, providing the first evidence for miRNA-directed RNAi therapy in AIDS [48]. Recently, we found that nascent mRNA-genomic DNA interactions can induce Id-miRNA production in mammalian cells [13]. These findings suggest that mammalian Pol-II RNA transcription and splicing-processing machineries are required for the release of intronic miRNA activities. Since there are more than 135 kinds of miRNA structures in mammals and many of them are functionally uncharacterized, the investigation of length requirement, structural determinant and mismatch tolerance of these miRNA-like molecules in the intron-derived gene silencing mechanism may reveal one of the major gene modulation systems for developmental regulation, intracellular immunity, heterochromatin inactivation and genomic evolution in eukaryotes.

We continue to explore the detailed pathway involved in the mechanism by which intron-derived miRNA represses gene expression. We are particularly interested in the requirement of multiple intracellular systems which remain to be determined. How do eukaryotic cells process a single-stranded intron-derived RNA to its active form of various hairpin constructs? Is this mediated by complementing-excision proteins or by Dicer-like endonucleases? Where is the intracellular compartment for this processing? How does Id-miRNA execute RNAi functions to silence specific gene expression? And how many sequences in the human genome can serve as a template for Id-miRNA generation?

Since Pol-II RNA transcription, splicing-processing and complementing-repairing machineries were involved in Id-miRNAs, a more thorough understanding of the intron-mediated RNAi mechanism must rely on a broad scope of research in the interactions among these systems. A comprehensive encyclopedia of intron-derived miRNA functions is essential to the complete application of such a mechanism to establish a better understanding of human biological processes, to predict potential disease risks, and to stimulate the development of new therapies and interventions for prevention and treatment of various diseases. Treatments based on such a cellular gene modulation system can advance current therapeutic design and provide a safer means for gene therapy since the Id-miRNA production is tightly

regulated by intracellular transcription and splicing machineries. Therefore, we have shown, for the first time, a new strategy for miRNA-mediated gene therapy in mammalian cells. The targeted gene domain subject to the therapy can be inserted into the non-snRNP-binding region of a gene intron, serving as a convenient tool for analysis of gene function and development of gene-specific therapeutics. This strategy may be useful for investigating the potential role of endogenous gene-homologous introns in regulating gene function and also prevention of undesired gene activities, steering cells away from malignancies due to oncogenes and viral genes.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

Pol-II	=	RNA polymerases type II
RNA	=	Ribonucleic acids
pre-mRNA	=	Precursor messenger RNA
snRNP	=	Small nuclear ribonucleoprotein particle
SS	=	Splice site
BrP	=	Branch point
PPT	=	Poly-pyrimidine tract
Ts	=	Termination codons
NMD	=	Non-sense mediated RNA decay
miRNA	=	MicroRNA
Id-miRNA	=	Intron-derived microRNA
stRNA	=	Short temporary RNA
shRNA	=	Small hairpin RNA
tncRNA	=	Tiny non-coding RNA
spRNA	=	Splicing-processed RNA
RNAi	=	RNA interference
RISC	=	RNA-induced silencing complex
SpRNAi	=	Splicing-competent RNA intron
rGFP	=	Mutated HcRed1 coral fluorescent protein gene
eGFP	=	Green fluorescent jellyfish protein gene
RdRp	=	RNA-directed RNA polymerase
XPC	=	Xeroderma pigmentosum group C syndrome
ITGb1	=	Integrin β1
HAART	=	Highly active antiretroviral therapy
HIV	=	Human immunodeficiency virus
AIDS	=	Acquired immunodeficiency syndrome

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